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L. EDELEN
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

INVENTORS / APPLICANTS

Given Name (last name, first name, and middle initial [if any])			Residence (City and State or Foreign Country)
NAKAMURA	Yusuke		Kanagawa, Japan
NAKAGAWA	Hidewaki		Tokyo, Japan
NAKATSURU	Syuichi		Saitama, Japan

TITLE OF THE INVENTION

PIN-PRC TRANSITION GENES

CORRESPONDENCE ADDRESS

Attorney Name: Firm Name and Address:	Ingrid A. Beattie; J.D., Ph.D. MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C. One Financial Center Boston, MA 02111
Telephone:	(617) 542-6000
Fax:	(617) 542-8841

ENCLOSED APPLICATION PARTS

- ☒ **Specification** — Number of Pages: 58
- ☒ **Sequence Listing** — Number of Pages: 14
- ☒ **Drawings** (☐ Formal; ☒ Informal) — Number of Sheets: 4
- ☐ **Other documents (specify):**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:

- ☒ No.
- ☐ Yes, the name of the U.S. Government agency and the Government contract number are:

METHOD OF PAYMENT

- ☒ A check in the amount of **$160.00** is enclosed to cover the filing fees of the Provisional application.
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- ☒ The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No. 50-0311, Reference No. 25371-028 PRO..

Respectfully submitted,

IABeattie

Date: February 27, 2004

Ingrid A. Beattie, No. 42,306
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.
Telephone: (617) 542-6000
Facsimile: (617) 542-2241
CUSTOMER NO. 30623

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NAKAMURA	Yusuke		Kanagawa, Japan
NAKAGAWA	Hidewaki		Tokyo, Japan
NAKATSURU	Syuichi		Saitama, Japan

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MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.
Telephone: (617) 542-6000
Facsimile: (617) 542-2241
CUSTOMER NO. 30623

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DESCRIPTION

PIN-PRC TRANSITION GENES

FIELD OF THE INVENTION

The invention relates to methods of diagnosing a predisposition to developing prostate cancer (PRC).

BACKGROUND OF THE INVENTION

Prostate cancer (PRC) is one of the most common malignancy in males and the second-leading cause of cancer-related deaths in the United States and Europe (Gronberg *et al.*, 2003). The testing for prostate specific antigen (PSA) in serum can detect early stage of PRC and it is now a gold standard to screen PRC in the high-risk population.

Incidence of prostate cancer is increasing steadily in developed countries according to the prevalence of Western–style diet and increasing number of senior population. Early diagnosis through serum testing for prostate specific antigen (PSA) provides an opportunity for curative surgery and has significantly improved the prognosis of prostate cancer, but up to 30% of patients treated with radical prostatectomy relapse their cancer (Han *et al.*, 2001). Most relapsed or advanced cancers respond to androgen ablation therapy because prostate cancer growth is initially androgen-dependent. However, they eventually progress to androgen-independent disease, at which point they are no longer responsive to androgen ablation therapy. The most serious clinical problem of prostate cancer is that androgen-independent prostate cancer is unresponsive to any other therapies (Gronberg, 2003), and establishing new therapies other than androgen ablation therapy against prostate cancer are a urgent issue for management of prostate cancer.

High-grade prostatic intraepithelial neoplasia (PIN) is widely accepted as the main premalignant lesion without invasion of the basal membrane of the acini, which has the potential to progress to invasive PRC (McNeal and Bostwick *et al.* 1986, DeMarzo *et al.* 2003, Abate-Shen *et al.* 2000, Montironi *et al.* 2002,). PIN does not significantly elevate

serum PSA concentration and cannot be detected by ultrasound.

High-grade PIN has a high predictive value as a marker for PRC, and its identification warrants repeat biopsy for concurrent or subsequent invasive PRC. Only prostate needle biopsy can recognize this minimal lesions and its identification warrants repeat biopsy for concurrent or subsequent invasive PRC (Bostwick 2000). Performing saturation prostate biopsies to rule out any coexistent prostate cancer followed by every 3-6 month serial repeated prostate biopsies is currently the only way in which to manage patients found to have high-grade PIN. But the reliability of this diagnosis is highly dependent on the technique of prostate needle biopsy, histological processing, and experience of reviewing pathologists (van der Kwast *et al.* 2003). They cannot perfectly discriminate PRC lesions from PRC nor identify the patients with invasive PRC among the high-risk people with PINs.

Hence accurate identification of PINs and PRC and understanding the prostatic carcinogenesis through PINs are important to avoid error in the diagnosis of invasive PRC and in patient management (Steiner 2001). However, the natural history of PINs and molecular mechanism of the putative transition form PINs to PRC reminds unclear and it is still controversial whether these PIN lesions without PRC should be treated or not.

cDNA microarray technologies have enabled to obtain comprehensive profiles of gene expression in normal and malignant cells, and compare the gene expression in malignant and corresponding normal cells (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61: 3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)). This approach enables to disclose the complex nature of cancer cells, and helps to understand the mechanism of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, Cell 103:311-20 (2000)). To disclose mechanisms underlying tumors from a genome-wide point of view, and discover target molecules for diagnosis and development

of novel therapeutic drugs, the present inventors have been analyzing the expression profiles of tumor cells using a cDNA microarray of 23040 genes (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61:3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)).

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnexyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita et al., Cancer Res 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int J Cancer 54: 177-80 (1993); Boon and van der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al., J Exp Med 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7 (1991)), gp100 (Kawakami et al., J Exp Med 180: 347-52 (1994)), SART (Shichijo et al., J Exp Med 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets

inducing cellular immune responses. Such gene products include p53 (Umano et al., Brit J Cancer 84: 1052-7 (2001)), HER2/neu (Tanaka et al., Brit J Cancer 84: 94-9 (2001)), CEA (Nukaya et al., Int J Cancer 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med 4: 321-7 (1998); Mukherji et al., Proc Natl Acad Sci USA 92: 8078-82 (1995); Hu et al., Cancer Res 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al.,J Exp Med 180: 347-52 (1994); Shichijo et al., J Exp Med 187: 277-88 (1998); Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997); Harris, J Natl Cancer Inst 88: 1442-5 (1996); Butterfield et al., Cancer Res 59: 3134-42 (1999); Vissers et al., Cancer Res 59: 5554-9 (1999); van der Burg et al., J Immunol 156: 3308-14 (1996); Tanaka et al., Cancer Res 57: 4465-8 (1997); Fujie et al., Int J Cancer 80: 169-72 (1999); Kikuchi et al., Int J Cancer 81: 459-66 (1999); Oiso et al., Int J Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or –A0201 restricted manner in ^{51}Cr-release assays (Kawano et al., Cance Res 60: 3550-8 (2000); Nishizaka et al., Cancer Res 60: 4830-7 (2000); Tamura et al., Jpn J Cancer Res 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J Immunol 155: 4307-12 (1995); Kubo et al., J Immunol 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-

affinity CTL in vitro usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7 (1996)).

SUMMARY OF THE INVENTION

The gene-expression profiles of cancer cells from 20 PRCs and 10 high-grade PINs were analyzed using cDNA microarray representing 23,040 genes coupled with laser microbeam microdissection (LMM) to characterize the molecular mechanisms involved in the putative transition from PINs to invasive PRC. By comparing expression patterns between cancer cells from diagnostic PRC patients and PIN cells purely selected with Laser Microdisection, 41 genes were identified as being up-regulated in PRC cells compared to in PIN cells, and 98 genes were identified as being down-regulated in PRC cells compared to in PIN cells. In addition, selection was made of candidate molecular markers with the potential of detecting cancer-related proteins in serum or sputum of patients, and discovered some potential targets for development of signal-suppressing strategies in human PRC.

Laser microdissection allows us to isolate pure cell populations and enables the precise evaluation (Emmert-Buck et al., 1996). Once isolated high-grade PINs without PRC are identified, treatment of high-grade PINs would appear to be of clinical benefit, and preventing from PINs to invasive PRC would reduce morbidity, enhance the quality of life, delay surgery or radiation, and increase the interval for surveillance requiring invasive procedures (Steiner *et al.* 2001, Nelson *et al.* 2001, Montironi *et al.* 2002). These data would provide important information on prostatic carcinogenesis and would be greatly useful to identify candidate genes whose products can be targeted for drug design for treatment and prevention of PRC.

The invention is based on the discovery of a pattern of gene expression correlated with PRC and PIN. The genes that are differentially expressed in PRC compared to PIN are collectively referred to herein as "PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins."

Accordingly, the invention features a method of diagnosing or determining a predisposition to developing PRC in a subject by determining an expression level of a PRC-associated gene in a patient derived biological sample, such as tissue sample. By PRC associated gene is meant a gene that is characterized by an expression level which differs in a cell obtained from a PRC cell compared to PIN cell. A PRC-associated gene includes for example PRC 1-139. An alteration, *e.g.*, increase or decrease of the level of expression of the gene compared to expression level of the gene in PIN indicates that the subject is at risk of developing PRC.

The invention also features a method of diagnosing or determining a predisposition to PRC in a subject by determining an expression level of EPHA4 in a patient derived biological sample, such as tissue sample. An alteration, *e.g.*, increase of the level of expression of EPHA4 compared to a normal control level indicates that the subject suffers from or is at risk of developing PRC.

By normal control level is meant a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from PRC and PIN. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A normal individual is one with no clinical symptoms of PRC and PIN.

An increase in the level of PRC 1-41 detected in a test sample compared to a level in PIN indicates the subject (from which the sample was obtained) is at risk of developing PRC. In contrast, a decrease in the level of PRC 42-139 detected in a test sample compared to a level in PIN indicates said subject is at risk of developing PRC.

Alternatively, expression of a panel of PRC-associated genes in the sample is compared to a PRC level of the same panel of genes. By PRC level is meant the expression profile of the PRC-associated genes found in a population suffering from PRC.

Gene expression is increased or decreased 10%, 25%, 50% compared to the level in PIN. Alternately, gene expression is increased or decreased 1, 2, 5 or more fold compared to the level in PIN. Expression is determined by detecting hybridization, *e.g.*, on an array, of a PRC-associated gene probe to a gene transcript of the patient-derived tissue sample.

The patient derived tissue sample is any tissue from a test subject, e.g., a patient

known to or suspected of having PRC. For example, the tissue contains an epithelial cell. For example, the tissue is an epithelial cell from prostate tissue.

The invention provides method for discriminating PRC form PINs and detect malignant PRC cells with high sensitivity using PRC 1-139. Especially, APOD and EphA4 are useful as specific markers for discriminating PRC from high-grade PINs.

The invention also provides a PRC reference expression profile of a gene expression level of two or more of PRC 1-139. Alternatively, the invention provides a PRC reference expression profile of the levels of expression two or more of PRC 1-41 or PRC 42-139.

The invention further provides methods of identifying an agent that inhibits or enhances the expression or activity of a PRC-associated gene,e.g PRC 1-139 by contacting a test cell expressing a PRC associated gene with a test agent and determining the expression level or activity of the PRC associated gene. The test cell is an epithelial cell such as an epithelial cell from prostate tissue. A decrease of the expresssion level or biological activity compared to that of the up-regulated marker gene in PRC indicates that the test agent is an inhibitor of expression or function of the PRC-associated gene and reduces a symptom of PRC, e.g, PRC 1-41. In the present invention, EphA4 (PRC 11) can be used as preferable up regulated marker gene. Alternatively, an increase of the expresssion level or biological activity compared to that of the down-regulated marker gene in PRC indicates that said test agent is an enhancer of expression or function of the PRC associated gene and reduces a symptom of PRC, e.g, PRC 42-139. Moreover, a decrease of the expresssion level or biological activity in the presence of the agent compared to that in the absence of the test agent indicates the agent is an inhibitor of an PRC associated upregulated gene and useful to inhibit PRC. Alternatively, an increase of the expresssion level or biological activity of the PRC-associated gene compared to that in the absence of the test agent indicates that the test agent augments expression or activity of the downregulated PRC associated gene.

The invention also provides a kit with a detection reagent which binds to two or more PRC polynucleotides or which binds to a gene product encoded by the nucleic acid sequences. Also provided is an array of nucleic acids that binds to two or more PRC nucleic acids.

The lists of the genes associated with malignant transformation also could provide with a number of information which is essential to establish novel chemo-preventive drugs for PRC transformation, and these chemo-preventive drugs could be treated effectively to the selected high-risk population of PRC, that is, those with high-grade PINs for the purpose of treating or preventing PRC.

Therapeutic methods include a method of treating or preventing PRC in a subject by administering to the subject an antisense composition. The antisense composition reduces the expression of a specific target gene, e.g., the antisense composition contains a nucleotide, which is complementary to a sequence selected from the group consisting of PRC 1-41. Another method includes the steps of administering to a subject an small interfering RNA (siRNA) composition. The siRNA composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-41. In yet another method, treatment or prevention of PRC in a subject is carried out by administering to a subject a ribozyme composition. The nucleic acid-specific ribozyme composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-41. Other therapeutic methods include those in which a subject is administered a compound that increases the expression of PRC 42-139 or activity of a polypeptide encoded by PRC 42-139. Furthermore, PRC can be treated by administering a protein encoded by PRC 42-139. The protein may be directly administered to the patient or, alternatively, may be expressed in vivo subsequent to being introduced into the patient, for example, by administering an expression vector or host cell carrying the down-regulated marker gene of interest. Suitable mechanisms for in vivo expression of a gene of interest are known in the art.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing PRC in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-41 or an immunologically active fragment such a polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response. For example, an immunologically active fragment at least 8 residues in length and stimulates an immune cell such as a T cell or a B cell. Immune cell stimulation is measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2), or production of an antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an illustration depicting the pathway for human prostate cancer progression. High-grade prostatic intraepithelial neoplasia (PIN) is widely accepted as the main premalignant lesion, which has the potential to progress to invasive PRC.

Figure 2 are photographs showing the results of immunohistochemical analysis of genes that were identified to be differentially expressed in the transition from PIN to PRC. Apolipoprotein D (APOD) was abundantly expressed in PRC cells, while PINs and normal prostatic epithelium (N) from the same patient showed no expression of APOD protein. The EphA4 protein was also strongly expressed in PRC cells, while PINs and normal prostatic epithelium (N) from the same patient showed no or very weak expression of EphA4 protein. The PRC, PIN and normal prostate epithelium were included on one prostate cancer tissue. Magnification, x200.

Figure 3. Northern blot analysis showed the expression pattern in normal adult tissues of EphA4. EphA4 is abundant only in adult testis, suggesting that targeting for EphA4 would be expected to lead less toxicity in human body.

Figure 4. Knocking-down endogenous EphA4 in prostate cancer cell line, PC3, by siRNA. (A) RT-PCR validated knockdown effect of EphA4 mRNA by transfection of siRNA expression vectors 1313si, but not by 198si, 468si1 and EGFPsi. 198si, 468si and 1313si were designed specifically for EphA4 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. β2-MG

was used to normalize input cDNA. (B) Colony formation assay showed drastic decrease of colony numbers in the cells one week after transfection with 1313si that were validated to knock down EphA4 effectively by RT-PCR. (C) MTT assay also showed drastic decreased number of the grown cells transfected with1313si, but not with 198si, 468si1 and EGFPsi

DETAILED DESCRIPTION

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acid sequences in epithelial cells of patients with PRC compared to expression in PIN cells. The differences in gene expression were identified by using a comprehensive cDNA microarray system.

The differentially expressed genes identified herein are used for diagnostic purposes as markers of predisposition to developing PRC and as gene targets, the expression of which is altered to treat or alleviate a symptom of PRC. The term "predisposition" as used herein indicates a potential to develop PRC from PIN. Predisposition can be diagnosed by measuring the expression levels of PRC associated genes which expression levels are altered in the transiton from PIN to PRC.

Alternatively, the differentially expressed genes within PIN and PRC identified herein are used for diagnostic purposes as markers to distinguish PRC from PIN and as gene targets, the expression of which is altered to treat or alleviate a symptom of PRC.

The genes whose expression levels are modulated (*i.e.*, increased or decreased) in PRC patients are summarized in Tables 1 and 2, and are collectively referred to herein as " PRC-associated genes ","PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins." Unless indicated otherwise, "PRC" is meant to refer to any of the sequences disclosed herein. (*e.g.*, PRC 1-139). The genes that have been previously described are presented along with a database accession number.

By measuring expression of the various genes in a sample of cells, a predisposition to developing PRC are diagnosed. Similarly, by measuring the expression of these genes in response to various agents, agents for treating PRC can be identified.

The invention involves determining (*e.g.*, measuring) the expression of at least one, and up to all the PRC sequences listed in Tables 1 and 2. Using sequence information provided by the GeneBank™ database entries for the known sequences the PRC associated genes are detected and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to PRC sequences, are used to construct probes for detecting PRC RNA sequences in, *e.g.*, northern blot hybridization analysis. Probes include at least 10, 20, 50, 100, 200 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the PRC nucleic acid in, *e.g*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction.

Expression level of one or more of the PRC-associated genes in the test cell population, *e.g.*, a patient derived tissues sample is then compared to expression levels of the some genes in a reference cell population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, PIN cells. The expression level of PRC 1-139 in the specimens from the test cell population and reference cell population may be determined at the same time. Alternatively, expression levels of the PRC 1-139 in reference cell population can be determined by a statistical method based on the results obtained by analyzing the expression level of the gene in specimens previously collected.

A pattern of gene expression in the test cell population compared to the reference cell population indicates a predisposition to developing PRC. When the expression level of the gene in test cell population does not fall within the range of reference cell population, the subject is judged to have high risk to develop PRC.

Moreover, if the reference cell population is made up of PRC cells, a similar gene expression profile between the test cell and the reference cell population indicates that the subject is judged to have high risk to develop PRC.

A level of expression of a PRC marker gene in a test cell population is considered altered in levels of expression if its expression level varies from the reference cell

population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold from the expression level of the corresponding PRC marker gene in the reference cell population.

Differential gene expression between a test cell population and a reference cell population is normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, a control nucleic acid is one which is known not to differ depending on the PRC or non-PRC state of the cell. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations. Control genes include β-actin, glyceraldehyde 3- phosphate dehydrogenase or ribosomal protein P1.

The test cell population is compared to multiple reference cell populations. Each of the multiple reference populations may differ in the known parameter. Thus, a test cell population may be compared to a second reference cell population known to contain, *e.g.*, PRC cells, as well as a second reference population known to contain, *e.g.*, PIN cells. The test cell is included in a tissue type or cell sample from a subject known to contain, or to be suspected of containing, PRC cells.

The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood, or serum). For example, the test cell is purified from a tissue. Preferably, the test cell population comprises a epithelial cell. The epithelial cell is from tissue known to be or suspected to be cancerous. Cells in the reference cell population are derived from a tissue type as similar to test cell. Optionally, the refernce cell poulation is a cell line, *e.g.* a PRC cell line (positive control)。 .Alternatively, the control cell population is derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

Expression of the genes disclosed herein is determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these nucleic acid sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed gene sequences. Expression is also determined at the protein level, *i.e.*, by measuring the

levels of polypeptides encoded by the gene products described herein, or biological activity thereof. Such methods are well known in the art and include, *e.g.*, immunoassays based on antibodies to proteins encoded by the genes. The biological activity of the proteins encoded by the genes are also well known.

Diagnosing PRC

A predisposition to developing PRC is diagnosed by measuring the expression level of one or more PRC polynucleotides from a test population of cells, (*i.e.*, a patient derived biological sample). Moreover, PRC or predisposition to developing PRC are diagnosed by measuring the expression level of EPHA4 from a test cell population. Preferably, the test cell population comprises an epithelial cell, e.g., a cell obtained from prostate tissue. Gene expression is also measured from blood or other bodily fluids such as urine. Other biological samples can be used for measuring the protein level. For example, the protein level in the blood, or serum derived from subject to be diagnosed can be measured by immunoassay or biological assay.

Expression of one or more of an PRC-associated gene, *e.g.*, PRC 1-139 is determined in the test cell or biological sample and compared to the expression level in reference cell. An increase or a decrease of the level of expression in the patient derived tissue sample of the PRC associated genes indicates that the subject is at risk of developing PRC. For example, an increase in expression of PRC 1-41 in the test population compared to the expression level in PIN indicates that the subject is at risk of developing PRC. Conversely, a decrease in expression of PRC 42-139 in the test population compared to the expression level in PIN indicates that the subject is at risk of developing PRC.

When the expression level of one or more of the PRC-associated genes are altered in the test population compared to the epression level in PIN indicates that the subject is at risk of developing PRC. For example, at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of PRC-associated genes (PRC 1-41 or PRC 42-139) are altered.

The expression levels of the PRC 1-139 in a particular specimen can be estimated by quantifying mRNA corresponding to or protein encoded by PRC 1-139. Quantification methods for mRNA are known to those skilled in the art. For example, the levels of mRNAs corresponding to the PRC 1-139 can be estimated by Northern blotting or RT-PCR. Since the nucleotide sequence of the PRC 1-139 have already been reported.

Anyone skilled in the art can design the nucleotide sequences for probes or primers to quantify the PRC 1-139.

Also the expression level of the PRC 1-139 can be analyzed based on the activity or quantity of protein encoded by the gene. A method for determining the quantity of the PRC 1-139 protein is shown in bellow. For example, immunoassay method is useful for the determination of the proteins in biological materials. Any biological materials can be used for the determination of the protein or it's activity. For example, blood sample is analyzed for estimation of the protein encoded by a serum marker. On the other hand, a suitable method can be selected for the determination of the activity of a protein encoded by the PRC 1-139 according to the activity of each protein to be analyzed.

In the present invention, a diagnostic agent for diagnosing predisposition to developing PRC, is also provided. The diagnostic agent of the present invention comprises a compound that binds to a polynucleotide or a polypeptide of the present invention. Preferably, an oligonucleotide that hybridizes to the polynucleotide of the PRC 1-41, or an antibody that binds to the polypeptide of the PRC 1-41 may be used as such a compound.

Identifying Agents that inhibit or enhance PRC-associated gene expression

An agent that inhibits the expression or activity of an PRC-associated gene is identified by contacting a test cell population expressing an PRC associated upregulated gene with a test agent and determining the expression level or activity of the PRC associated gene. A decrease of expression or activity in the presence of the agent compared to the level or activity in the absence of the test agent indicates the agent is an inhibitor of an PRC associated upregulated gene and useful to inhibit PRC.

Alternatively, an agent that enhances the expression or activity of an PRC downregulated associated gene is identified by contacting a test cell population expressing an PRC associated gene with a test agent and determining the expression level or activity of the PRC associated downregulated gene. An increase of expression or activity of the PRC-associated gene compared to the expression or activity in the absence of the test agent indicates that the test agent augments expression or activity of the downregulated PRC associated gene.

The test cell population is any cell expressing the PRC-associated genes. For example, the test cell population contains an epithelial cell, such as a cell is or derived

from prostate. For example, the test cell is immortalized cell line derived from a PRC cell. Alternatively, the test cell is a cell, which has been transfected with a PRC-associated gene or which has been transfected with a regulatory sequence (*e.g.* promoter sequence) from a PRC-associated gene operably linked to a reporter gene.

Assessing efficacy of treatment of PRC in a subject

The differentially expressed PRC-associated gene identified herein also allow for the course of treatment of PRC to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for PRC. If desired, test cell populations are obtained from the subject at various time points before, during, or after treatment. Expression of one or more of the PRC-associated gene, in the cell population is then determined and compared to a reference cell population which includes cells whose PRC state is known. The reference cells have not been exposed to the treatment.

If the reference cell population contains no PRC cells, a similarity in expression between PRC-associated gene in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between PRC -associated gene in the test population and PIN reference cell population indicates the a less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to a reduction in expression of a pathologically upregulated gene, increase in expression of a pathologically down-regulated gene or a decrease in size, prevalence, or metastatic potential of PRC in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents a PRC from forming or retards, prevents, or alleviates a symptom of clinical PRC. Assesment of prostate tumors are made using standard clinical protocols.

Efficaciousness is determined in association with any known method for diagnosing or treating PRC. PRC is diagnosed for example, by identifying symptomatic anomalies, *e.g.*, urinary symptoms such as difficulty in starting or stopping the stream, dysuria, frequency, or hematuria.

Selecting a therapeutic agent for treating PRC that is appropriate for a particular individual

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to

act as an inhibitor of PRC can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of an PRC state to a gene expression pattern characteristic of a non-PRC state. Accordingly, the differentially expressed PRC-associated gene disclosed herein allow for a putative therapeutic or prophylactic inhibitor of PRC to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable PRC inhibitor in the subject.

To identify a inhibitor of PRC, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of PRC 1-139 genes is determined.

The test cell population contains a PRC cell expressing a PRC associated gene. Preferably, the test cell is an epithelial cell. For example a test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test sample is measured and compared to one or more reference profiles, *e.g.* ,PIN reference expression profile.

A decrease in expression of one or more of PRC 1-41 or an increase in expression of one or more of PRC 42-139 in a test cell population relative to a reference cell population containing PRC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agents are immunomodulatory agents.

Screening assays for identifying therapeutic agents

The differentially expressed genes disclosed herein can also be used to identify candidate therapeutic agents for treating PRC. The method is based on screening a candidate therapeutic agent to determine if it converts an expression profile of PRC 1-139 characteristic of an PRC state to a pattern indicative of a PIN state.

In the present invention, PRC 1-139 are useful for screening of therapeutic agent for treating or preventing PRC.

In the method, a cell is exposed to a test agent or a combination of test agents (sequentially or consequentially) and the expression of one or more PRC 1-139 in the cell is measured. The expression profile of the PRC-associated gene in the test population is compared to expression level of the PRC-associated gene in a reference cell population that is not exposed to the test agent.

An agent effective in stimulating expression of underexpressed genes, or in suppressing expression of overexpressed genes is deemed to lead to a clinical benefit such compounds are further tested for the ability to prevent PRC in animals or test subjects.

In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment or prevention of PRC. As discussed in detail above, by controlling the expression levels or activities of marker genes, one can control the onset and progression of PRC. Thus, candidate agents, which are potential targets in the treatment or prevention of PRC, can be identified through screenings that use the expression levels and activities of marker genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-139, ;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 1-139; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-41, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 42-139.

Cells expressing a marker gene include, for example, cell lines established from PRC; such cells can be used for the above screening of the present invention.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-139;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-41 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 42-139 in comparison with the biological activity detected in the absence of the test compound.

A protein required for the screening can be obtained as a recombinant protein using the nucleotide sequence of the marker gene. Based on the information of the marker gene, one skilled in the art can select any biological activity of the protein as an index for screening and a measurement method based on the selected biological activity.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 1-139

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 1-41 as compared to a level in PIN, or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 42-139, as compared to a level in PIN.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

In the method for screening of the present invention, EphA4 (PRC 11) can be used as preferable upregulated marker gene. Furthermore, we here identified a tyrosine kinase receptor, *EphA4*, as an over-expressed gene specifically in invasive prostate cancer, not in non-invasive precursor PINs (prostatic intraepitherial neoplasia), by using genome-wide cDNA microarray combined with laser microbeam microdissection. The cDNA microarray and immunohistochemistry demonstrated that EphA4 was over-expressed specifically in invasive prostate cancer cells, not in PINs, and Northern blot analysis showed its restricted expression in adult testis. The knocking-down effect by siRNA specific to *EphA4* resulted in drastic suppression of prostate cancer cell growth. These findings suggest that EphA4 was associated with growth and motility of invasive prostate cancer cells and this tyrosine kinase receptor, EphA4, can be promising molecular targets for novel prostate cancer therapy without drastic side effect. Accordingly, an agent that inhibits tyrosine kinase activity of EphA4 is useful for therapeutic agent, treating, or prevention of PRC. Thus, present invention provides a method for screening an agent for treating or prevention of PRC comprises, for example the following step:

a) contacting a test compound with a polypeptide encoded by EPHA4;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide in comparison with the biological activity detected in the absence of the test compound.

In the present invention, biological activity of EphA4 is preferably tyrosine kinase activity. The skilled artisan can estimate tyrosine kinase activity. For example, contacting a cell expressing EphA4 with test compound at presence of [γ-^{32}P]-ATP. Then, phosphorylated protein by tyrosine kinase activity of EphA4 are determined. For detection of the phosphorylated protein, SDS-PAGE or immunoprecipitation can be used. Furthermore, an antibody recognizes phosphrylated tyrosine residue can be used for phosphrylated protein level.

The compound isolated by the screening is a candidate for drugs that inhibit or enhance the activity of the protein encoded by marker genes and can be applied to the treatment or prevention of PRC.

Moreover, compound in which a part of the structure of the compound inhibiting or enhancing the activity of proteins encoded by marker genes is converted by addition,

deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

When administrating the compound isolated by the method of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can also be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol and phenol; and an anti-

oxidant. The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the pharmaceutical composition of the present inevntion to patients, for example as intraarterial, intravenous, or percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable metod of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to the body-weight, age, and symptoms of the patient but one skilled in the art can suitably select them.

For example, although the dose of a compound that binds to the protein of the present invention and regulates its activity depends on the symptoms, the dose is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kgs of body-weight.

Assessing the prognosis of a subject with PRC

Also provided is a method of assessing the prognosis of a subject with PRC by comparing the expression of one or more PRC-associated gene s in a test cell population to the expression of the genes in a reference cell population derived from patients over a spectrum of disease stages. By comparing gene expression of one or more PRC-associated gene in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

A decrease in expression of one or more of PRC 42-139 compared to a expression in PIN or an increase of expression of one or more of PRC 1-41 compared to a expression

in PIN indicates less favorable prognosis. An increase in expression of one or more of PRC 42-139 indicates a more favorable prognosis, and a decrease in expression of PRC 1-41 indicates a more favorable prognosis for the subject.

Kits

The invention also includes an PRC-detection reagent, e.g., a nucleic acid that specifically binds to or identifies one or more PRC nucleic acids such as oligonucleotide sequences, which are complementary to a portion of an PRC nucleic acid or antibodies which bind to proteins encoded by an PRC nucleic acid. The reagents are packaged together in the form of a kit. The reagents are packaged in separate containers, e.g., a nucleic acid or antibody (either bound to a solid matrix or packaged separately with reagents for binding them to the matrix) , a control reagent (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay are included in the kit. The assay format of the kit is a Northern hybridization or a sandwich ELISA known in the art.

For example, PRC detection reagent, is immobilized on a solid matrix such as a porous strip to form at least one PRC detection site. The measurement or detection region of the porous strip may include a plurality of sites containing a nucleic acid. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, *i.e.*, a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of PRC present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acid sequences. The nucleic acids on the array specifically identify one or more nucleic acids represented by PRC 1-139. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-139 are identified by virtue if the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305.

Arrays and pluralities

The invention also includes a nucleic acid substrate array comprising one or more nucleic acid. The nucleic acids on the array specifically corresponds to one or more nucleic acid sequences represented by PRC 1-139. The level expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-139 are identified by detecting nucleic acid binding to the array.

The invention also includes an isolated plurality (*i.e.*, a mixture if two or more nucleic acids) of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acids represented by PRC 1-139. In various embodiments, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-139.

Methods of inhibiting PRC

The invention provides a method for treating or alleviating a symptom of PRC in a subject by decreasing expression or activity of PRC 1-41 or increasing expression or activity of PRC 42-139. Therapeutic compounds are administered prophylactically or therapeutically to subject suffering from at risk of (or susceptible to) developing PRC. Such subjects are identified using standard clinical methods or by detecting an aberrant level of expression or activity of (*e.g.*, PRC 1-139). Therapeutic agents include inhibitors of cell cycle regulation, cell proliferation, and protein kinase activity.

The therapeutic method includes increasing the expression, or function, or both of one or more gene products of genes whose expression is decreased ("underexpressed genes") in PRC cell relative toPIN cells of the same tissue type from which the PRC or PIN cells are derived. In these methods, the subject is treated with an effective amount of a compound, which increases the amount of one of more of the underexpressed genes in the subject. Administration can be systemic or local. Therapeutic compounds include a polypeptide product of an underexpressed gene, or a biologically active fragment thereof a nucleic acid encoding an underexpressed gene and having expression control elements permitting expression in the PRC cells; for example an agent which increases the level of expression of such gene endogenous to the PRC cells (i.e., which up-regulates expression of the underexpressed gene or genes). Administration of such compounds counter the

effects of aberrantly-under expressed of the gene or genes in the subject's prostate cells and improves the clinical condition of the subject.

The method also includes decreasing the expression, or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("overexpressed gene") in. Expression is inhibited in any of several ways known in the art. For example, expression is inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the overexpressed gene or genes, e.g., an antisense oligonucleotide or small interfering RNA which disrupts expression of the overexpressed gene or genes.

Alternatively, function of one or more gene products of the overexpressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the overexpressed gene product or gene products.

As noted above, antisense nucleic acids corresponding to the nucleotide sequence of PRC 1-41 can be used to reduce the expression level of the PRC 1-41. Antisense nucleic acids corresponding to PRC 1-41 that are up-regulated in PRC are useful for the treatment PRC. Specifically, the antisense nucleic acids of the present invention may act by binding to the PRC 1-41 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or inhibiting the expression of proteins encoded by a nucleic acid selected from the group consisting of the PRC 1-41, finally inhibiting the function of the proteins . The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid derivatives of the present invention act on cells producing the proteins encoded by marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the

degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid derivative of the present invention can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples are, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the invention inhibit the expression of the protein of the invention and is thereby useful for suppressing the biological activity of a protein of the invention. Also, expression-inhibitors, comprising the antisense nucleic acids of the invention, are useful since they can inhibit the biological activity of a protein of the invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

Also, a siRNA against marker gene can be used to reduce the expression level of the marker gene. By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. In the context of the present invention, the siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence against an upregulated marker gene, such as PRC 1-41. The siRNA is constructed such that a single transcript has both the sense and

complementary antisense sequences from the target gene, e.g., a hairpin.

Binding of the siRNA to a transcript corresponding to one of the PRC 1-41 in the target cell results in a reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring the transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , 25 nucleotides in length.

The nucleotide sequence of the siRNAs were designed using a siRNA design computer program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with the binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene for evaluation

The antisense oligonucleotide or siRNA of the invention inhibit the expression of the polypeptide of the invention and is thereby useful for suppressing the biological activity of the polypeptide of the invention. Also, expression-inhibitors, comprising the antisense oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the biological activity of the polypeptide of the invention. Therefore, a composition comprising the antisense oligonucleotide or siRNA of the present invention are useful in treating or preventing a PRC.

Alternatively, function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure, that interacts (i.e., binds) only with the antigen that was used for synthesizing the antibody (i.e., the up-regulated marker gene product) or with an antigen closely related to it. Furthermore, an antibody may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston J. S. et al. Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co M. S. et al. J. Immunol. 152:2968-2976 (1994); Better M. and Horwitz A. H. Methods Enzymol. 178:476-496 (1989); Pluckthun A. and Skerra A. Methods Enzymol. 178:497-515 (1989); Lamoyi E. Methods Enzymol. 121:652-663 (1986); Rousseaux J. et al. Methods Enzymol. 121:663-669 (1986); Bird R. E. and Walker B. W. Trends Biotechnol. 9:132-137 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from a nonhuman antibody, the frame work region (FR) derived from a human antibody, and the constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells

have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res. 2001 Oct;7(10):2958-70. Review.; Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001 Mar 15;344(11):783-92.; Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, Driessen C, Rudiger T, Muller-Hermelink K, Diehl V, Engert A. Treatment of relapsed CD20+ Hodgkin lymphoma with the monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial of the German Hodgkin Lymphoma Study Group. Blood. 2003 Jan 15;101(2):420-424.; Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S and Bhalla KN. (2000). Blood, 96, 2246-2253.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002). Oncology, 63 Suppl 1, 47-56.; Klejman A, Rushen L, Morrione A, Slupianek A and Skorski T. (2002). Oncogene, 21, 5868-5876.). Therefore, future cancer treatments will probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods are performed *ex vivo* or *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression or activity of the differentially expressed genes.

Diseases and disorders that are characterized by increased levels or biological activity of the genes relative to the levels or activities in PIN may be treated with therapeutics that antagonize (*i.e.*, reduce or inhibit) activity of the overexpressed gene or

genes. Therapeutics that antagonize activity are administered therapeutically or prophylactically.

Therapeutics that may be utilized include, *e.g.*, (*i*) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the underexpressed gene or genes; (*ii*) antibodies to the overexpressed gene or genes; (*iii*) nucleic acids encoding the underexpressed gene or genes; (*iv*) antisense nucleic acids or nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of one or more overexpressed genes); (*v*) small interfering RNA (siRNA); or (*vi*) modulators (*i.e.*, inhibitors, agonists and antagonists that alter the interaction between an over/underexpressed polypeptide and its binding partner. The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, *e.g.*, Capecchi, *Science* 244: 1288-1292 1989)

Diseases and disorders that are characterized by decreased levels or biological activity relative to the levels or activities in PIN may be treated with therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods include contacting a cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. An agent that modulates protein activity includes a nucleic acid or a protein, a naturally-occurring

cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, the agent stimulates one or more protein activities of one or more of a differentially under-expressed gene.

The present invention also relates to a method of treating or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-41 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. An administration of the polypeptide induce an anti-tumor immunity in a subject. To inducing anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-41 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide is administered. The polypeptide or the immunologically active fragments thereof are useful as vaccines against PRC. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell recepor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against PRC refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by a nucleic acid selected from the group consisting of PRC 1-41 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against PRC cells expressing PRC 1-41. Thus, the present invention also encompasses method of inducing anti-tumor immunity using the polypeptides. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing in vivo or in vitro the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ^{3}H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that the it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL against tumors by contacting with the polypeptides are useful as vaccines against tumors. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods

for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of PRC. Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer. Decrease in mortality of individuals having cancer, decrease of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analysis.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such.

The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide ex vivo, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, comprising a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

Pharmaceutical compositions for inhibiting PRC

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also include powders, granules or solutions, suspensions or emulsions. The active ingredient os optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded

tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on each day of the month.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol

spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims. The following examples illustrate the identification and characterization of genes differentially expressed in PRC or PIN cells.

Example 1: General Methods

Patients and tissue samples

Tissue samples were obtained with informed consent from 26 cancer patients undergoing radical prostatectomy. All surgical specimens were at clinical stages T2a-T3a with or without N1, and their Gleason scores were 5-9. Histopathological diagnoses were made by a single pathologist before LMM. All samples were embedded in TissueTek OCT medium (Sakura, Tokyo, Japan) immediately after surgical resection and stored at $-80^{\circ}C$ until use. From among the 26 resected tissues, 20 cancers and 10 high-grade PINs had sufficient amounts and quality of RNA for microarray analysis.

Laser microbeam microdissection and T7-based RNA amplification

LMM and T7-based RNA amplification were performed as described previously. Prostate tumor cells and normal prostatic ductal epithelial cells were isolated selectively using the EZ cut system with a pulsed ultraviolet narrow beam-focus laser (SL Microtest GmbH, Germany) in accordance with the manufacturer's protocols. After DNase treatment, total RNAs were subjected to two rounds of T7-based amplification, which yielded 50–100 μg of aRNA from each sample. Then 2.5 μg aliquots of aRNA from PRC or PIN cells and from normal prostatic ductal epithelial cells were labeled by reverse transcription with Cy5-dCTP (tumor cells) or Cy3-dCTP (normal cells) (Amersham Biosciences, Buckinghamshire, UK), as described previously (Ono *et al.* 2000).

cDNA microarray analysis and acquisition of data

We fabricated a genome-wide cDNA microarray with 23,040 cDNAs selected from the UniGene database (build #131) of the National Center for Biotechnology Information (NCBI). Construction, hybridization, washing, and scanning were carried out according to methods described previously (Ono *et al.* 2000). Signal intensities of Cy3 and Cy5 from the 23,040 spots were quantified and analyzed by substituting backgrounds, using ArrayVision software (Imaging Research, Inc., St. Catharines, Ontario, Canada). Subsequently, the fluorescent intensities of Cy5 (tumor) and Cy3 (control) for each target spot were adjusted so that the mean Cy3/Cy5 ratio of 52 housekeeping genes was equal to one. Because data derived from low signal intensities are less reliable, we determined a cut-off value on each slide (Ono *et al.* 2000) and excluded genes from further analysis when both Cy3 and Cy5 dyes yielded signal intensities lower than the cut-off. For other genes, we calculated the Cy5/Cy3 ratio using the raw data of each sample.

Identification of genes that were up- or down-regulated from PINs to PRC

We identified genes with changed expression in 20 PRC and 10 PINs according to the following criteria: 1) genes for which we were able to obtain expression data in more than 50% of the cases examined; and 2) genes whose expression ratio was more than 3.0 in prostate cancers and between 0.5 and 2.0 in PINs (defined as up-regulated genes) or genes whose expression ratio was less than 0.33 in cancers and between 0.5 and 2.0 in PINs (defined as down-regulated genes) in more than 50% of informative cases.

Immunohistochemistry

Formalin-fixed and paraffin-embedded prostatic tumor sections were immunostained

using a mouse anti-APOD (apolipoprotein D) monoclonal antibody (NEOMARKERS, Fremont, CA) or a rabbit anti-EPHA4 (EphA4) polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) for APOD or EPHA4 expression. Prostate cancer tissues included PRC cells, PIN cells and normal protstatic epithelium heterogeneously. Deparaffinized tissue sections were placed in 10 mM citrate buffer, pH 6.0, and heated to 108°C in an autoclave for 15 minutes for antigen retrieval. Sections were incubated with a 1:10 dilution or a 1:100 dilution of primary antibody for APOD or EPHA4, respectively, in a humidity chamber for an hour at room temperature, and developed with peroxidase labeled-dextran polymer followed by diaminobenzidine (DAKO Envision Plus System; DAKO Corporation, Carpinteria, CA). Sections were counterstained with hematoxylin. For negative controls, primary antibody was omitted.

Northern-blot analysis. Human multiple-tissue Northern blots (Clontech, Palo Alto, CA) were hybridized with a [α^{32}P] dCTP-labeled PCR product of EphA4. The 1014-bp PCR products were prepared by RT-PCR using primers:
5'-GAAGGCGTGGTCACTAAATGTAA-3'(SEQ ID NO:3) and
5'- TTTAATTTCAGAGGGCGAAGAC-3' (SEQ ID NO:4). Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at -80°C for 7 days.

siRNA-expressing constructs and colony formation / MTT assay. We used siRNA-expression vector (psiU6BX) for RNAi effect to the target genes. The U6 promoter was cloned into the upstream of the gene specific sequence (19nt sequence from the target transcript separated by a short spacer TTCAAGAGA (SEQ ID NO:5) from the reverse complement of the same sequence) and five thymidines as a termination signal, furthermore neo cassette was integrated to become resistant to Geneticin (Sigma). The target sequences for *EphA4* are
5'-TCCGAACCTACCAAGTGTG-3' (SEQ ID NO:6) (198si),
5'-TCATGAAGCTGAACACCGA-3' (SEQ ID NO:7) (468si) and
5'-GCAGCACCATCATCCATTG-3' (SEQ ID NO:8) (1313si), and

5'-GAAGCAGCACGACTTCTTC-3' (SEQ ID NO:9) (EGFPsi) as a negative control. The target sequences were designed against full length sequence of EphA4. The nucleotide sequence of EphA4 and amino acid sequence encoded by the nucleotide sequence were shown as SEQ ID NO:1 and SEQ ID NO:2, respectivery (GenBank Accession No. NM_004438). PC3 prostate cancer cell lines were plated onto 10-cm dishes (5×10^5 cells/dish) and transfected with psiU6BX containing EGFP target sequence (*EGFP*) and psiU6BX containing target sequence using Lipofectamine 2000 (Invitrogen) according to manufacture's instruction. Cells were selected by 500 mg/ml Geneticin for one week, and preliminary cells were harvested 48 hours after transfection and analyzed by RT-PCR to validate knockdown effect on *EphA4*. The primers of RT-PCR were the same ones described above. These cells were also stained by Giemsa solution and performed MTT assay to evaluate the colony formation and the cell number, respectively.

Example 2: Identification of genes up- or down-regulated during malignant transformation from PINs to prostate cancers

We focused on differential expression patterns between PINs and PRC to search for genes likely to be involved in the transition from non-invasive precursor PINs to malignant cancers (Figure 1). Comparing the expression profiles of 20 PRC with those of 10 PINs, we identified 41 up-regulated genes (Table 1) and 98 down-regulated genes (Table 2). The list included *POV1, CDKN2C, EPHA4, APOD FASN*, and *VWF* as up-regulated, and *ITGB2, LAMB2, PLAU* and *TIMP1* as down-regulated; the altered genes might be involved with cell adhesion or motility in invasive PRC cells. *EPHA4* is one of the receptor tyrosine kinase receptors and is likely to play a critical role of neuronal circuit development and angiogenesis by regulating cell shape and motility; and its overexpression in PRC is likely to be associated with PRC cell motility (Kullander *et al.* 2002). Some of the later are associated with cell adhesion and proteinase activity, suggesting that their expression changes may contribute to the invasive phenotype by abolishing ductal structures during the transition from PIN to PRC.

Table 1 Up-regulated genes in the transition from PIN to PRC

	Accession No.	Hs.	Symbol	Title
function known				
1	X12433	99364	ABHD2	abhydrolase domain containing 2
2	AF039018	135281	ALP	alpha-actinin-2-associated LIM protein
3	H61951	12152	APMCF1	APMCF1 protein
4	J02611	75736	APOD	apolipoprotein D
5	AA633487	108708	CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta
6	AI357641	4854	CDKN2C	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)
7	NM_004938	153924	DAPK1	death-associated protein kinase 1
8	NM_004405	419	DLX2	distal-less homeo box 2
9	T78186	241565	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
10	W94051	336678	DTNA	dystrobrevin, alpha
11	L36645 NM_004438	73964	EPHA4	EphA4
12	M16967	30054	F5	coagulation factor V (proaccelerin, labile factor)
13	U29344	83190	FASN	fatty acid synthase
14	AF100143	6540	FGF13	fibroblast growth factor 13
15	D14446	107	FGL1	fibrinogen-like 1
16	BE747327	7644	HIST1H1C	histone 1, H1c
17	BG163483	76907	HSPC002	HSPC002 protein
18	AF064493	4980	LDB2	LIM domain binding 2
19	U21128	79914	LUM	lumican
20	U07620	151051	MAPK10	mitogen-activated protein kinase 10
21	NM_002465	169849	MYBPC1	myosin binding protein C, slow type
22	AI767296	123655	NPR3	natriuretic peptide receptor C/guanylate cyclase C
23	X76770	49007	PAPOLA	poly(A) polymerase alpha
24	AF045584	18910	POV1	prostate cancer overexpressed gene 1
25	AI298501	21192	SDK1	sidekick homolog 1 (chicken)
26	AB020335	181300	SEL1L	sel-1 suppressor of lin-12-like (C. elegans)
27	BE735822	75069	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)
28	N21096	99291	STXBP6	syntaxin binding protein 6 (amisyn)

29	AF091395	367689	TRIO	triple functional domain (PTPRF interacting)
30	AK000235	31608	TRPM4	transient receptor potential cation channel, subfamily M, member 4
31	NM_000552	110802	VWF	von Willebrand factor
function unknown				
32	N66442	135971		ESTs
33	BE274422	380933		Homo sapiens mRNA; cDNA DKFZp586O1224
34	AI304487	171443		Homo sapiens, clone IMAGE:3354344, mRNA, partial cds
35	AA830405	403857		Homo sapiens, clone IMAGE:5932767, mRNA
36	D14657	81892	KIAA0101	KIAA0101 gene product
37	W63676	356547	LOC129642	hypothetical protein BC016005
38	AW295100	5957	LOC201562	hypothetical protein LOC201562
39	AL137707	103422	LOC220115	hypothetical protein LOC220115
40	AI057614	293845	LOC89944	hypothetical protein BC008326
41	AW972144	422113	MGC30006	hypothetical protein MGC30006

Table 2 Down-regulated genes in the transition from PIN to PRC

	Accession No.	Hs.	Symbol	Title
function known				
42	AI827230	374481	APCDD1	adenomatosis polyposis coli down-regulated 1
43	BF965257	74120	APM2	adipose specific 2
44	AA156854	114309	APOL1	apolipoprotein L, 1
45	NM_004024	460	ATF3	activating transcription factor 3
46	M94345	82422	CAPG	capping protein (actin filament), gelsolin-like
47	AF035752	139851	CAV2	caveolin 2
48	D13639	75586	CCND2	cyclin D2
49	M16445	89476	CD2	CD2 antigen (p50), sheep red blood cell receptor
50	AI750036	22116	CDC14B	CDC14 cell division cycle 14 homolog B (S. cerevisiae)
51	AK021865	173380	CKIP-1	CK2 interacting protein 1; HQ0024c protein
52	X15880	108885	COL6A1	collagen, type VI, alpha 1
53	L16510	297939	CTSB	cathepsin B

54	U03688	154654	CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1
55	M62401	82568	CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1
56	X90579	166079	CYP3A5P2	cytochrome P450, family 3, subfamily A, polypeptide 5 pseudogene 2
57	X93920	180383	DUSP6	dual specificity phosphatase 6
58	NM_001421	151139	ELF4	E74-like factor 4 (ets domain transcription factor)
59	AF275945	116651	EVA1	epithelial V-like antigen 1
60	AW300770	61265	FAM3D	family with sequence similarity 3, member D
61	D84239	111732	FCGBP	Fc fragment of IgG binding protein
62	AF182316	234680	FER1L3	fer-1-like 3, myoferlin (C. elegans)
63	NM_001924	80409	GADD45A	growth arrest and DNA-damage-inducible, alpha
64	W91908	6079	GALNAC4S-6ST	B cell RAG associated protein
65	AA666119	92287	GBP3	guanylate binding protein 3
66	NM_000165	74471	GJA1	gap junction protein, alpha 1, 43kDa (connexin 43)
67	J03004	77269	GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
68	J03817	301961	GSTM1	glutathione S-transferase M1
69	M33906	198253	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1
70	NM_018950	110309	HLA-F	major histocompatibility complex, class I, F
71	U26726	1376	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2
72	BF793633	180919	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein

73	AV646610	34853	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
74	M15395	83968	ITGB2	integrin, beta 2
75	U25138	93841	KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1
76	AB012955	129867	KIP2	DNA-dependent protein kinase catalytic subunit-interacting protein 2
77	X72760	90291	LAMB2	laminin, beta 2 (laminin S)
78	Y00711	234489	LDHB	lactate dehydrogenase B
79	M36682	621	LGALS3	lectin, galactoside-binding, soluble, 3 (galectin 3)
80	L13210	79339	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
81	X03444	377973	LMNA	lamin A/C
82	AA779709	7457	MAGE-E1	MAGE-E1 protein
83	L08895	78995	MEF2C	MADS box transcription enhancer factor 2, polypeptide C
84	AF017418	104105	MEIS2	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)
85	AF203032	198760	NEFH	neurofilament, heavy polypeptide 200kDa
86	M12267	75485	OAT	ornithine aminotransferase (gyrate atrophy)
87	AW051593	189999	P2RY5	purinergic receptor P2Y, G-protein coupled, 5
88	BG028573	64056	PAK1	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)
89	BF969355	8364	PDK4	pyruvate dehydrogenase kinase, isoenzyme 4
90	AA253194	303125	PIGPC1	p53-induced protein PIGPC1
91	M22430	76422	PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)
92	D00244	77274	PLAU	plasminogen activator, urokinase
93	X56134	297753	RPLP2	ribosomal protein, large P2

94	W73992	132792	SDCCAG43	serologically defined colon cancer antigen 43
95	AW965789	66450	SENP1	sentrin/SUMO-specific protease
96	AF029082	184510	SFN	stratifin
97	U44403	75367	SLA	Src-like-adaptor
98	AV705470	380991	SNF1LK	SNF1-like kinase
99	Y08110	101657	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing
100	BE439695	160483	STOM	stomatin
101	AB042646	94785	TGIF2	TGFB-induced factor 2 (TALE family homeobox)
102	NM_003241	2387	TGM4	transglutaminase 4 (prostate)
103	U21847	82173	TIEG	TGFB inducible early growth response
104	M12670	5831	TIMP1	tissue inhibitor of metalloproteinase 1
105	AA837002	9741	TJP4	tight junction protein 4 (peripheral)
106	M35252	84072	TM4SF3	transmembrane 4 superfamily member 3
107	M19309	73980	TNNT1	troponin T1, skeletal, slow
108	W72411	137569	TP73L	tumor protein p73-like
109	H99016	171501	USP11	ubiquitin specific protease 11
110	AF077197	74669	VAMP5	vesicle-associated membrane protein 5 (myobrevin)
111	AW137980	115659	VIK	vav-1 interacting Kruppel-like protein
112	D88154	103665	VILL	villin-like
113	M92843	343586	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
114	BF055342	326801	ZNF6	zinc finger protein 6 (CMPX1)
function unknown				
115	AI769569	112472		ESTs
116	AW510657	156044		ESTs
117	BF111819	21470		ESTs
118	T79422	119237		ESTs
119	AI304862	12867		ESTs
120	AA705222	119880		ESTs

121	AA768607	122926		ESTs
122	AI052358	131741		ESTs
123	AW888225	250723		ESTs, Weakly similar to hypothetical protein FLJ20378 [Homo sapiens]
124	BF223679	118747		Homo sapiens cDNA FLJ33407 fis, clone BRACE2010535.
125	AI821113	292781		Homo sapiens cDNA FLJ36327 fis, clone THYMU2005748.
126	AL360198	22870		Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 34988.
127	AL050204	28540		Homo sapiens mRNA; cDNA DKFZp586F1223 (from clone DKFZp586F1223)
128	AV733210	367688		Homo sapiens, clone IMAGE:4794726, mRNA
129	U57961	181304	13CDNA73	hypothetical protein CG003
130	AL050289	7446	C6orf4	chromosome 6 open reading frame 4
131	AW956111	79404	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence
132	AK001021	22505	FLJ10159	hypothetical protein FLJ10159
133	R43725	98927	FLJ13993	hypothetical protein FLJ13993
134	D42047	82432	KIAA0089	KIAA0089 protein
135	NM_014766	75137	KIAA0193	KIAA0193 gene product
136	AA921341	3610	KIAA0205	KIAA0205 gene product
137	AB007903	113082	KIAA0443	KIAA0443 gene product
138	BF431643	15420	KIAA1500	KIAA1500 protein
139	AA706316	32343	ZD52F10	hypothetical gene ZD52F10

Example 3: Immunohistochemistry

To validate the gene expression pattern in the transition from PIN to PRC, we performed immunohistochemical analysis of the genes differentially expressed in the

transition from PIN to PRC in our data. In general, prostate cancer tissues includes PRC cells, PIN cells and normal prostatic epithelium heterogenously, and we compared the staining pattern of each kinds of cells associated with prostatic carcinogenesis on the same tissues from the same patient. As shown in Figure 2, apolipoprotein D (APOD) was abundantly expressed in PRC cells while PINs and normal prostatic epithelium from the same patient had no or very weak expression of APOD protein. The EphA4 protein was also strongly expressed in PRC cells while PINs and normal prostatic epithelium from the same patient had no or very weak expression of EphA4 protein. The results implicate this expression profile analysis is highly reliable.

Example 4

we focused on *EphA4* because EphA4 is one of the receptors with tyrosine kinase activity and an ideal molecule target for drug design and antibody therapy against cancer. Now a number of tyrosine kinase inhibitors are on clinical trial for cancer treatment, including EGFR (epidermal growth factor receptor) inhibitors, PDGFR inhibitor, and VEGF (vascular endothelial growth factor) inhibitors (Dancey and Sausville *et al.*, 2003, Morgan *et al.*, 2003). In addition, trastuzumab (Herceptin), a humanized monoclonal antibody against a tyrosine kinase receptor ERBB2/Her2 (epidermal growth factor receptor 2), is effective for subsets of metastatic breast cancer with HER2 over-expressed (Dancey and Sausville *et al.*, 2003). These tyrosine kinase receptors as drug targets for cancer can be approached by both small molecules and antibody strategy, and it is obvious that they have most potentials as molecular targets for cancer therapy.

EphA4 is one of the receptor with tyrosine kinase activity and their functions with their ephrin ligands are best studies in the nervous system, where Eph receptors and ephrin molecules are involved in patterning the developing hindbrain, axon pathfinding and guiding neural crest cell migration (Dodelet *et al.*, 2000, Kurai and Pasquale, 2003). These molecules also regulate embryonic vascular development and there are some reports about the association of Eph/ephrin with tumor angiogenesis (Gale and Yancopoulos, 1999, Dodelet *et al.*, 2000). The Eph receptor family consists of 13 members and their ligands, ephirins, are divided into two subclasses, the A-subclass (A1-A5) and the B-subclass (B1-

B3). The receptors are divided on the basis of sequence similarity and ligand affinity into A--subclass (EphA1-A8), and B-subclass (EphB1-B4, B6). A-type receptors typically bind to most or all A-type ligands, and B-type receptors bind to most or all B-type ligands, with the exception of EphA4 that can bind both A-type and most B-type ligands (Dodelet *et al.*, 2000, Kurai and Pasquale, 2003). In prostate cancer tissues, the ligand of EphA4 is unknown. Northern blot analysis showed that EphA4 was abundant in testis, not in central nervous system and other major organs (Figure 3). Recently the antibody targeting against other Eph receptor family member, EphA2 that is also over-expressed in several cancers, was reported to inhibit breast cancer cell growth *in vitro* and *in vivo* (Carles-Kinch *et al.*, 2002, Coffman *et al.*, 2003). However, EphA2 is expressed ubiquitously in adult tissues, indicating much more possibility of toxicity in treatment of antibody therapy. Considering its tyrosine kinase activity, membrane localization and its restricted expression pattern, EphA4 is one the most ideal molecular targets for prostate cancer.

Example 5:*Growth suppression mediated by siRNA in prostate cancer cell lines*

To investigate the growth or survival effect on prostate cancer of *EphA4*, we knocked down their endogenous expression specifically by mammalian vector-based RNA interference (RNAi) technique. The transfection of the siRNA-producing vectors resulted in reduction of the endogenous expression in some designed siRNA for *EphA4* (Fig 4A). The knocking-down effect by the siRNA on the transcript of *EphA4* resulted in drastic growth suppression in colony formation assay and MTT assay (Fig 4B and 4C). These finding strongly suggested that overexpression of *EphA4* in prostate cancercells were associated with cancer cell growth and they were promising molecular targets of prostate cancer therapy by which these molecules can be blocked or knocked down.

In conclusion, we identified EphA4, a tyrosine kinase receptor over-expressed in prostate cancer cells, not in non-invasive precursor PINs, and it is likely to be associated with cancer cell growth, suggested this tyrosine kinase receptor are an ideal molecular targets of small molecules or antibodies for prostate cancer treatment.

Industrial Applicability

The gene-expression analysis of PRC and PIN described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a

subset of these differentially expressed genes, the present invention provides a molecular diagnostic markers for diagnosing a predisposition to developing PRC.

The methods described herein are also useful in the identification of additional molecular targets for prevention, and treatment of PRC. The data reported herein add to a comprehensive understanding of PRC, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of prostatic tumorigenesis, and provide indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of PRC.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

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CLAIMS

1. A method of diagnosing a predisposition to developing PRC in a subject, comprising determining a level of expression of a PRC-associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to expression level of said gene in PIN indicates that said subject is at risk of developing PRC.

2. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 1-41, wherein an increase in said level compared to a level in PIN indicates said subject is at risk of developing PRC.

3. The method of claim 2, wherein said increase is at least 10% greater than said level in PIN.

4. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 42-139, wherein a decrease in said level compared to a level in PIN indicates said subject is at risk of developing PRC.

5. The method of claim 4, wherein said decrease is at least 10% lower than said level in PIN.

6. The method of claim 1, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.

7. The method of claim 1, wherein the expression level is determined by any one method select from group consisting of:
(a) detecting the mRNA of the PRC -associated genes,
(b) detecting the protein encoded by the PRC -associated genes, and
(c) detecting the biological activity of the protein encoded by the PRC -associated genes.

8. The method of claim 1, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-derived biological sample.

9. The method of claim 8, wherein said hybridization step is carried out on a DNA array.

10. The method of claim 1, wherein said biological sample comprises an epithelial cell.

11. The method of claim 1, wherein said biological sample comprises prostate cancer cell.

12. The method of claim 8, wherein said biological sample comprises an epithelial cell from a PRC.

13. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 1-139.

14. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 1-41.

15. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 42-139.

16. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by PRC 1-139;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

17. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 1-139; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-41, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 42-139.

18. The method of claim 17, wherein said test cell comprises a prostate cancer cell.

19. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by selected from the group consisting of PRC 1-139;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by PRC 1-41 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by PRC 42-139 in comparison with the biological activity detected in the absence of the test compound.

20. A method of screening for compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 1-139

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 1-41, as compared to a level in PIN or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 42-139, as compared to a level in PIN.

21. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-139.

22. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-139.

23. A method of treating or preventing PRC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 1-41.

24. A method of treating or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 1-

41.

25. A method of treating or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-41.

26. A method of treating or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-41 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

27. A method of treating or preventing PRC in a subject comprising administering to said subject a compoud that increases the expression or activity of PRC 42-139.

28. A method of treating or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 16-20.

29. A method of treating or preventing PRC in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 42-139, or polypeptide encoded by thereof.

30. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 1-41 as an active ingredient, and a pharmaceutically acceptable carrier.

31. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-41 as an active ingredient, and a pharmaceutically acceptable carrier.

32. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 16-20 as an active ingredient, and a pharmaceutically acceptable carrier.

33. A method of diagnosing PRC or a predisposition to developing PRC in a subject,

comprising determining a level of expression of EPHA4 in a patient derived biological sample, wherein an increase of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PRC.

34. The method of claim 33, wherein said increase is at least 10% greater than said normal control level.

35. The method of claim 33, wherein the expression level is determined by any one method select from group consisting of:

(a) detecting the mRNA of EPHA4,

(b) detecting the protein encoded by EPHA4, and

(c) detecting the biological activity of the protein encoded by EPHA4,

36. The method of claim 33, wherein said level of expression is determined by detecting hybridization of EPHA4 probe to a gene transcript of said patient-derived biological sample.

37. The method of claim 36, wherein said hybridization step is carried out on a DNA array.

38. The method of claim 33, wherein said biological sample comprises an epithelial cell.

39. The method of claim 33, wherein said biological sample comprises PRC cell.

40. The method of claim 39, wherein said biological sample comprises an epithelial cell from a PRC.

41. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by EPHA4;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

42. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing EPHA4; and

b) selecting a compound that reduces the expression.

43. The method of claim 42, wherein said test cell comprises a prostate cancer cell.

44. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by EPHA4;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide in comparison with the biological activity detected in the absence of the test compound.

45. The amethod of claim 44, wherein the biological activity is tyrosine kinase activity.

46. A method of screening for compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a cell into which a vector comprising the transcriptional regulatory region of EPHA4 genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced,

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene, as compared to a level in the absence of the test compound.

47. A method of treating or preventing PRC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence of EPHA4.

48. A method of treating or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of EPHA4.

49. The method of claim 48, wherein said siRNA comprises the nucleotide sequence of SEQ ID NO: 8 as the target sequence.

50. A method of treating or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by EPHA4.

51. A method of treating or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by EPHA4 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

52. A method of treating or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 41-46.

53. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a EPHA4 as an active ingredient, and a pharmaceutically acceptable carrier.

54. The composition of claim 53, wherein said small interfering RNA comprises the nucleotide sequence of SEQ ID NO: SEQ ID NO: 8 as the target sequence.

55. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by EPHA4 as an active ingredient, and a pharmaceutically acceptable carrier.

56. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 41-46 as an active ingredient, and a pharmaceutically acceptable carrier.

57. The small interfering RNA, wherein the sense strand thereof comprises the nucleotide sequence of SEQ ID NO: 8 as the target sequence and less than 75 nucleotides in length.

Abstract

Objective methods for diagnosing a predisposition to developing prostate cancer (PRC) are described herein. In one embodiment, the diagnostic method involves the determining a expression level of PRC -associated gene that discriminate between PRC and PIN. The present invention further provides methods of screening for therapeutic agents useful in the treatment of PRC, methods of treating PRC.

Fig. 1

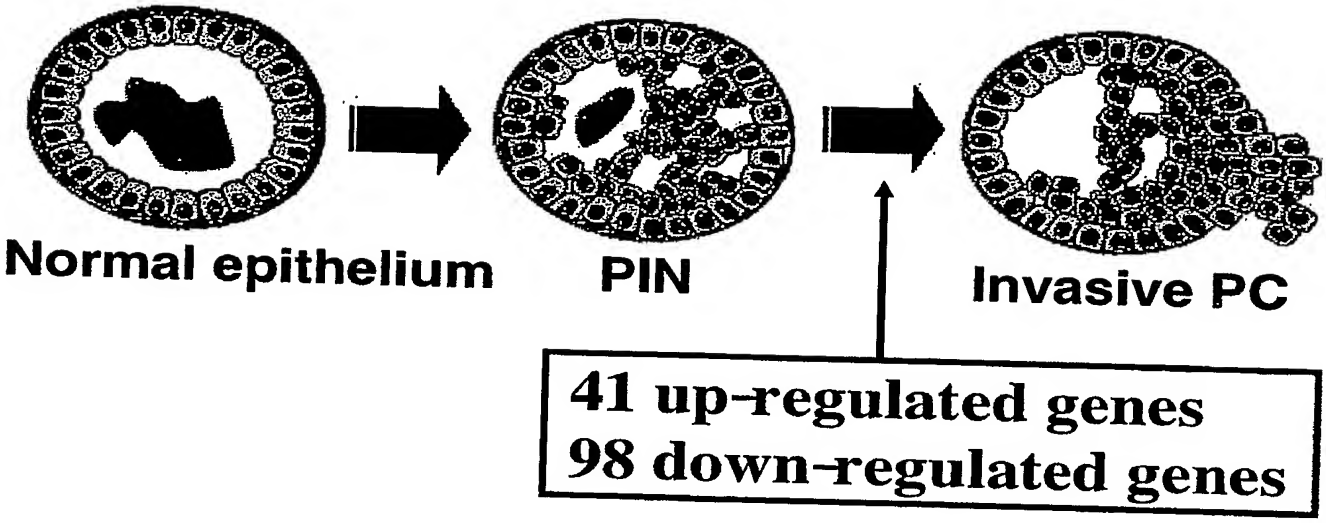


Fig. 2

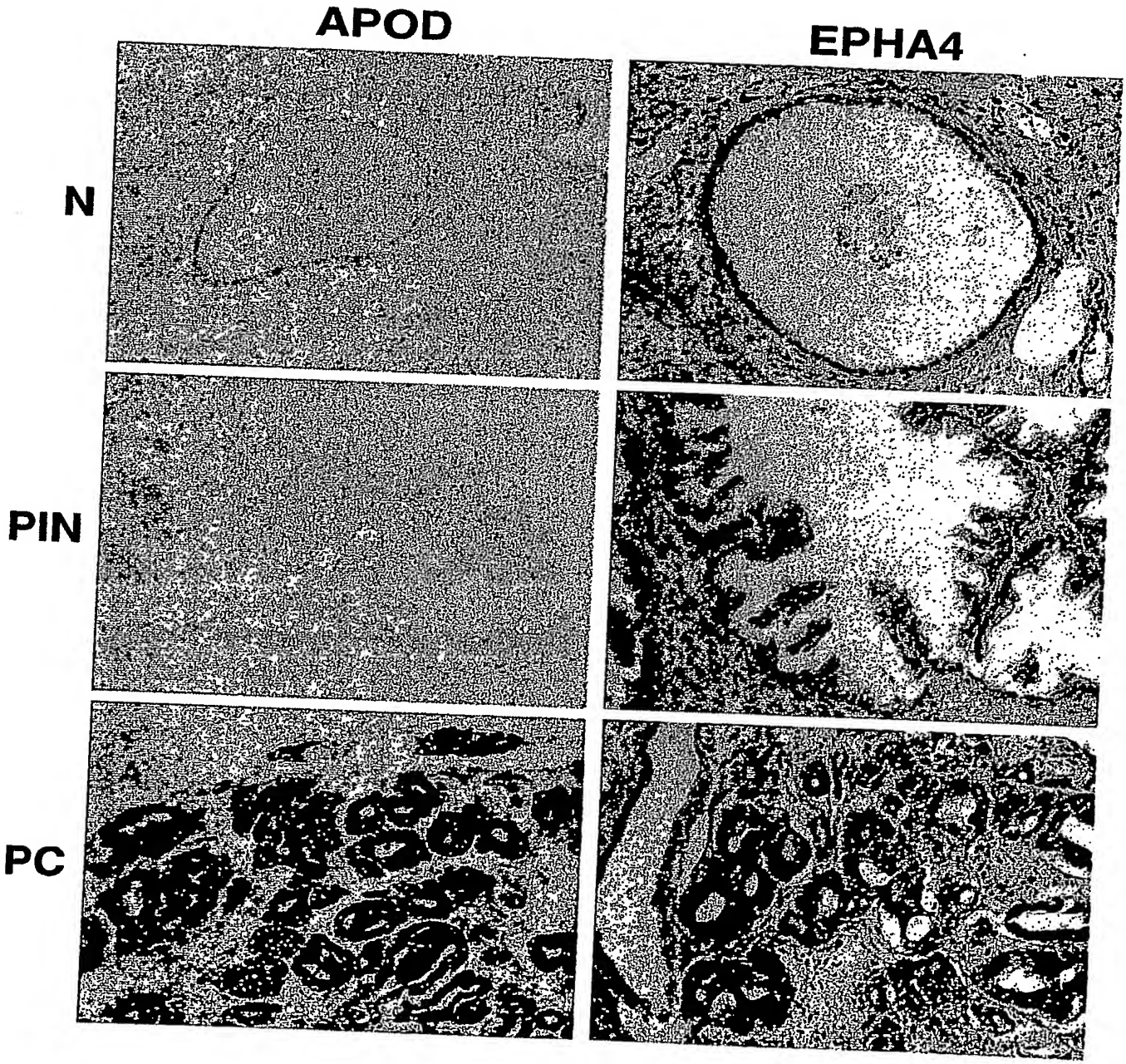


Fig. 3

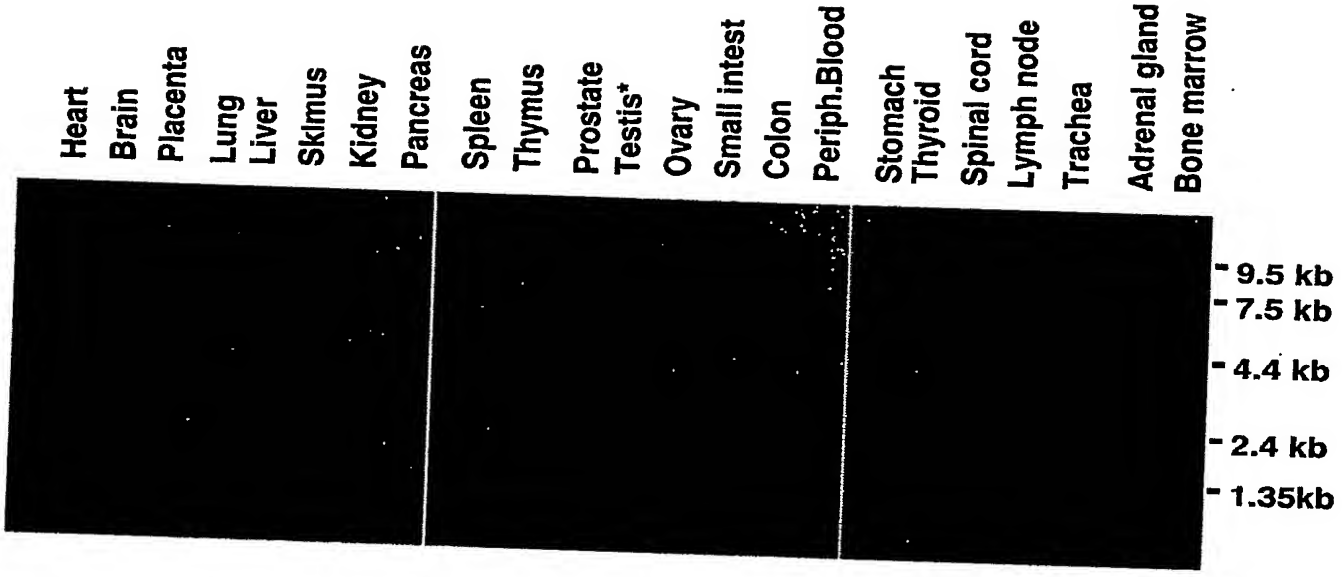
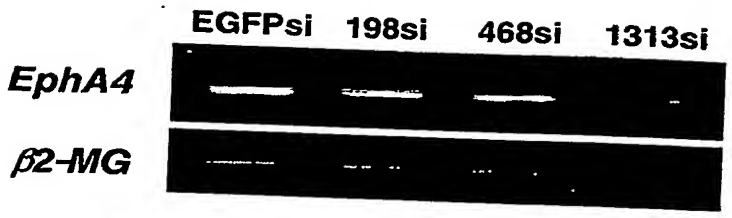


Fig. 4

(A)



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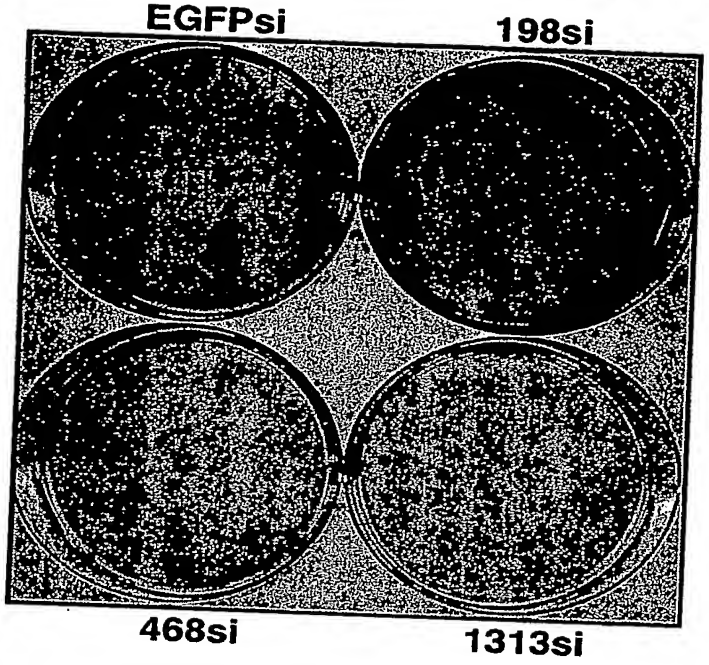

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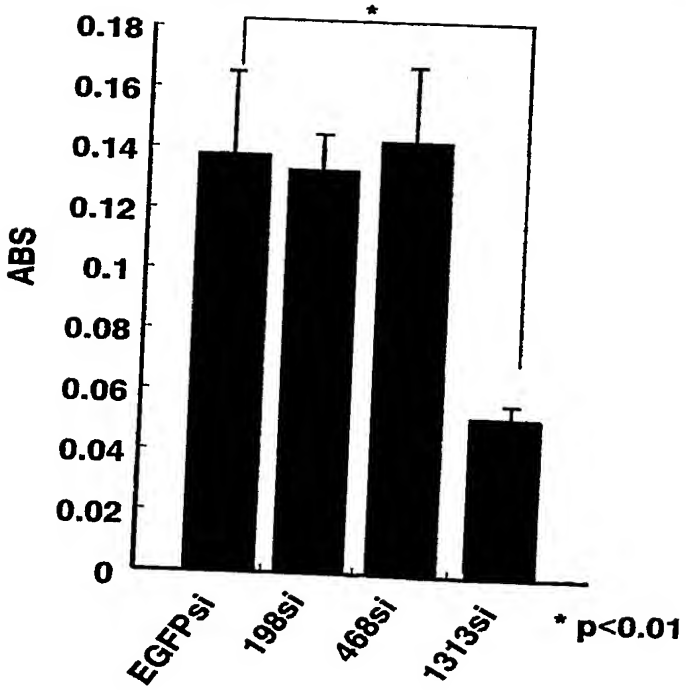

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5                   10                  15                  20

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Thr Gly Ser Arg Val Tyr Pro Ala Asn Glu Val Thr Leu Leu Asp Ser
                25                  30                  35

aga tct gtt cag gga gaa ctt ggg tgg ata gca agc cct ctg gaa gga          198
Arg Ser Val Gln Gly Glu Leu Gly Trp Ile Ala Ser Pro Leu Glu Gly
            40                  45                  50

ggg tgg gag gaa gtg agt atc atg gat gaa aaa aat aca cca atc cga          246
Gly Trp Glu Glu Val Ser Ile Met Asp Glu Lys Asn Thr Pro Ile Arg
        55                  60                  65

acc tac caa gtg tgc aat gtg atg gaa ccc agc cag aat aac tgg cta          294
Thr Tyr Gln Val Cys Asn Val Met Glu Pro Ser Gln Asn Asn Trp Leu
    70                  75                  80
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cga act gat tgg atc acc cga gaa ggg gct cag agg gtg tat att gag      342
Arg Thr Asp Trp Ile Thr Arg Glu Gly Ala Gln Arg Val Tyr Ile Glu
85                  90                  95                  100

att aaa ttc acc ttg agg gac tgc aat agt ctt ccg ggc gtc atg ggg      390
Ile Lys Phe Thr Leu Arg Asp Cys Asn Ser Leu Pro Gly Val Met Gly
                105                 110                 115

act tgc aag gag acg ttt aac ctg tac tac tat gaa tca gac aac gac      438
Thr Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Tyr Glu Ser Asp Asn Asp
            120                 125                 130

aaa gag cgt ttc atc aga gag aac cag ttt gtc aaa att gac acc att      486
Lys Glu Arg Phe Ile Arg Glu Asn Gln Phe Val Lys Ile Asp Thr Ile
        135                 140                 145

gct gct gat gag agc ttc acc caa gtg gac att ggt gac aga atc atg      534
Ala Ala Asp Glu Ser Phe Thr Gln Val Asp Ile Gly Asp Arg Ile Met
    150                 155                 160

aag ctg aac acc gag atc cgg gat gta ggg cca tta agc aaa aag ggg      582
Lys Leu Asn Thr Glu Ile Arg Asp Val Gly Pro Leu Ser Lys Lys Gly
165                 170                 175                 180

ttt tac ctg gct ttt cag gat gtg ggg gcc tgc atc gcc ctg gta tca      630
Phe Tyr Leu Ala Phe Gln Asp Val Gly Ala Cys Ile Ala Leu Val Ser
                185                 190                 195

gtc cgt gtg ttc tat aaa aag tgt cca ctc aca gtc cgc aat ctg gcc      678
Val Arg Val Phe Tyr Lys Lys Cys Pro Leu Thr Val Arg Asn Leu Ala
            200                 205                 210

cag ttt cct gac acc atc aca ggg gct gat acg tct tcc ctg gtg gaa      726
Gln Phe Pro Asp Thr Ile Thr Gly Ala Asp Thr Ser Ser Leu Val Glu
        215                 220                 225

gtt cga ggc tcc tgt gtc aac aac tca gaa gag aaa gat gtg cca aaa      774
Val Arg Gly Ser Cys Val Asn Asn Ser Glu Glu Lys Asp Val Pro Lys
    230                 235                 240

atg tac tgt ggg gca gat ggt gaa tgg ctg gta ccc att ggc aac tgc      822
Met Tyr Cys Gly Ala Asp Gly Glu Trp Leu Val Pro Ile Gly Asn Cys
245                 250                 255                 260

cta tgc aac gct ggg cat gag gag cgg agc gga gaa tgc caa gct tgc      870
Leu Cys Asn Ala Gly His Glu Glu Arg Ser Gly Glu Cys Gln Ala Cys
                265                 270                 275
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aaa att gga tat tac aag gct ctc tcc acg gat gcc acc tgt gcc aag      918
Lys Ile Gly Tyr Tyr Lys Ala Leu Ser Thr Asp Ala Thr Cys Ala Lys
            280                 285                 290

tgc cca ccc cac agc tac tct gtc tgg gaa gga gcc acc tcg tgc acc      966
Cys Pro Pro His Ser Tyr Ser Val Trp Glu Gly Ala Thr Ser Cys Thr
        295                 300                 305

tgt gac cga ggc ttt ttc aga gct gac aac gat gct gcc tct atg ccc     1014
Cys Asp Arg Gly Phe Phe Arg Ala Asp Asn Asp Ala Ala Ser Met Pro
    310                 315                 320

tgc acc cgt cca cca tct gct ccc ctg aac ttg att tca aat gtc aac     1062
Cys Thr Arg Pro Pro Ser Ala Pro Leu Asn Leu Ile Ser Asn Val Asn
325                 330                 335                 340

gag aca tct gtg aac ttg gaa tgg agt agc cct cag aat aca ggt ggc     1110
Glu Thr Ser Val Asn Leu Glu Trp Ser Ser Pro Gln Asn Thr Gly Gly
                345                 350                 355

cgc cag gac att tcc tat aat gtg gta tgc aag aaa tgt gga gct ggt     1158
Arg Gln Asp Ile Ser Tyr Asn Val Val Cys Lys Lys Cys Gly Ala Gly
            360                 365                 370

gac ccc agc aag tgc cga ccc tgt gga agt ggg gtc cac tac acc cca     1206
Asp Pro Ser Lys Cys Arg Pro Cys Gly Ser Gly Val His Tyr Thr Pro
        375                 380                 385

cag cag aat ggc ttg aag acc acc aaa gtc tcc atc act gac ctc cta     1254
Gln Gln Asn Gly Leu Lys Thr Thr Lys Val Ser Ile Thr Asp Leu Leu
    390                 395                 400

gct cat acc aat tac acc ttt gaa atc tgg gct gtg aat gga gtg tcc     1302
Ala His Thr Asn Tyr Thr Phe Glu Ile Trp Ala Val Asn Gly Val Ser
405                 410                 415                 420

aaa tat aac cct aac cca gac caa tca gtt tct gtc act gtg acc acc     1350
Lys Tyr Asn Pro Asn Pro Asp Gln Ser Val Ser Val Thr Val Thr Thr
                425                 430                 435

aac caa gca gca cca tca tcc att gct ttg gtc cag gct aaa gaa gtc     1398
Asn Gln Ala Ala Pro Ser Ser Ile Ala Leu Val Gln Ala Lys Glu Val
            440                 445                 450

aca aga tac agt gtg gca ctg gct tgg ctg gaa cca gat cgg ccc aat     1446
Thr Arg Tyr Ser Val Ala Leu Ala Trp Leu Glu Pro Asp Arg Pro Asn
        455                 460                 465
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ggg gta atc ctg gaa tat gaa gtc aag tat tat gag aag gat cag aat      1494
Gly Val Ile Leu Glu Tyr Glu Val Lys Tyr Tyr Glu Lys Asp Gln Asn
    470                 475                 480

gag cga agc tat cgt ata gtt cgg aca gct gcc agg aac aca gat atc      1542
Glu Arg Ser Tyr Arg Ile Val Arg Thr Ala Ala Arg Asn Thr Asp Ile
485                 490                 495                 500

aaa ggc ctg aac cct ctc act tcc tat gtt ttc cac gtg cga gcc agg      1590
Lys Gly Leu Asn Pro Leu Thr Ser Tyr Val Phe His Val Arg Ala Arg
                505                 510                 515

aca gca gct ggc tat gga gac ttc agt gag ccc ttg gag gtt aca acc      1638
Thr Ala Ala Gly Tyr Gly Asp Phe Ser Glu Pro Leu Glu Val Thr Thr
            520                 525                 530

aac aca gtg cct tcc cgg atc att gga gat ggg gct aac tcc aca gtc      1686
Asn Thr Val Pro Ser Arg Ile Ile Gly Asp Gly Ala Asn Ser Thr Val
        535                 540                 545

ctt ctg gtc tct gtc tcg ggc agt gtg gtg ctg gtg gta att ctc att      1734
Leu Leu Val Ser Val Ser Gly Ser Val Val Leu Val Val Ile Leu Ile
    550                 555                 560

gca gct ttt gtc atc agc cgg aga cgg agt aaa tac agt aaa gcc aaa      1782
Ala Ala Phe Val Ile Ser Arg Arg Arg Ser Lys Tyr Ser Lys Ala Lys
565                 570                 575                 580

caa gaa gcg gat gaa gag aaa cat ttg aat caa ggt gta aga aca tat      1830
Gln Glu Ala Asp Glu Glu Lys His Leu Asn Gln Gly Val Arg Thr Tyr
                585                 590                 595

gtg gac ccc ttt acg tac gaa gat ccc aac caa gca gtg cga gag ttt      1878
Val Asp Pro Phe Thr Tyr Glu Asp Pro Asn Gln Ala Val Arg Glu Phe
            600                 605                 610

gcc aaa gaa att gac gca tcc tgc att aag att gaa aaa gtt ata gga      1926
Ala Lys Glu Ile Asp Ala Ser Cys Ile Lys Ile Glu Lys Val Ile Gly
        615                 620                 625

gtt ggt gaa ttt ggt gag gta tgc agt ggg cgt ctc aaa gtg cct ggc      1974
Val Gly Glu Phe Gly Glu Val Cys Ser Gly Arg Leu Lys Val Pro Gly
    630                 635                 640

aag aga gag atc tgt gtg gct atc aag act ctg aaa gct ggt tat aca      2022
Lys Arg Glu Ile Cys Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr
645                 650                 655                 660
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gac aaa cag agg aga gac ttc ctg agt gag gcc agc atc atg gga cag      2070
Asp Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser Ile Met Gly Gln
                665                 670                 675

ttt gac cat ccg aac atc att cac ttg gaa ggc gtg gtc act aaa tgt      2118
Phe Asp His Pro Asn Ile Ile His Leu Glu Gly Val Val Thr Lys Cys
            680                 685                 690

aaa cca gta atg atc ata aca gag tac atg gag aat ggc tcc ttg gat      2166
Lys Pro Val Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ser Leu Asp
        695                 700                 705

gca ttc ctc agg aaa aat gat ggc aga ttt aca gtc att cag ctg gtg      2214
Ala Phe Leu Arg Lys Asn Asp Gly Arg Phe Thr Val Ile Gln Leu Val
    710                 715                 720

ggc atg ctt cgt ggc att ggg tct ggg atg aag tat tta tct gat atg      2262
Gly Met Leu Arg Gly Ile Gly Ser Gly Met Lys Tyr Leu Ser Asp Met
725                 730                 735                 740

agc tat gtg cat cgt gat ctg gcc gca cgg aac atc ctg gtg aac agc      2310
Ser Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser
                745                 750                 755

aac ttg gtc tgc aaa gtg tct gat ttt ggc atg tcc cga gtg ctt gag      2358
Asn Leu Val Cys Lys Val Ser Asp Phe Gly Met Ser Arg Val Leu Glu
            760                 765                 770

gat gat ccg gaa gca gct tac acc acc agg ggt ggc aag att cct atc      2406
Asp Asp Pro Glu Ala Ala Tyr Thr Thr Arg Gly Gly Lys Ile Pro Ile
        775                 780                 785

cgg tgg act gcg cca gaa gca att gcc tat cgt aaa ttc aca tca gca      2454
Arg Trp Thr Ala Pro Glu Ala Ile Ala Tyr Arg Lys Phe Thr Ser Ala
    790                 795                 800

agt gat gta tgg agc tat gga atc gtt atg tgg gaa gtg atg tcg tac      2502
Ser Asp Val Trp Ser Tyr Gly Ile Val Met Trp Glu Val Met Ser Tyr
805                 810                 815                 820

ggg gag agg ccc tat tgg gat atg tcc aat caa gat gtg att aaa gcc      2550
Gly Glu Arg Pro Tyr Trp Asp Met Ser Asn Gln Asp Val Ile Lys Ala
                825                 830                 835

att gag gaa ggc tat cgg tta ccc cct cca atg gac tgc ccc att gcg      2598
Ile Glu Glu Gly Tyr Arg Leu Pro Pro Pro Met Asp Cys Pro Ile Ala
            840                 845                 850
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ctc cac cag ctg atg cta gac tgc tgg cag aag gag agg agc gac agg      2646
Leu His Gln Leu Met Leu Asp Cys Trp Gln Lys Glu Arg Ser Asp Arg
        855                 860                 865

cct aaa ttt ggg cag att gtc aac atg ttg gac aaa ctc atc cgc aac      2694
Pro Lys Phe Gly Gln Ile Val Asn Met Leu Asp Lys Leu Ile Arg Asn
    870                 875                 880

ccc aac agc ttg aag agg aca ggg acg gag agc tcc aga cct aac act      2742
Pro Asn Ser Leu Lys Arg Thr Gly Thr Glu Ser Ser Arg Pro Asn Thr
885                 890                 895                 900

gcc ttg ttg gat cca agc tcc cct gaa ttc tct gct gtg gta tca gtg      2790
Ala Leu Leu Asp Pro Ser Ser Pro Glu Phe Ser Ala Val Val Ser Val
                905                 910                 915

ggc gat tgg ctc cag gcc att aaa atg gac cgg tat aag gat aac ttc      2838
Gly Asp Trp Leu Gln Ala Ile Lys Met Asp Arg Tyr Lys Asp Asn Phe
            920                 925                 930

aca gct gct ggt tat acc aca cta gag gct gtg gtg cac gtg aac cag      2886
Thr Ala Ala Gly Tyr Thr Thr Leu Glu Ala Val Val His Val Asn Gln
        935                 940                 945

gag gac ctg gca aga att ggt atc aca gcc atc acg cac cag aat aag      2934
Glu Asp Leu Ala Arg Ile Gly Ile Thr Ala Ile Thr His Gln Asn Lys
    950                 955                 960

att ttg agc agt gtc cag gca atg cga acc caa atg cag cag atg cac      2982
Ile Leu Ser Ser Val Gln Ala Met Arg Thr Gln Met Gln Gln Met His
965                 970                 975                 980

ggc aga atg gtt ccc gtc tga gccagtactg aataaactca aaactcttga         3033
Gly Arg Met Val Pro Val
                985

aattagttta cctcatccat gcactttaat tgaagaactg cacttttttt acttcgtctt    3093

cgccctctga aattaaagaa atgaaaaaaa aaaacaatat ctgcagcgtt gcttggtgca    3153

cagattgctg aaactgtggg gcttacagaa atgactgccg gtcatttgaa tgagacctgg    3213

aacaaatcgt ttctcagaag tacttttctg ttcatcacca gtctgtaaaa tacatgtacc    3273

tatagaaata gaacactgcc tctgagtttt gatgctgtat ttgctgccag acactgagct    3333

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Cys Asp Ala Val Thr Gly Ser Arg Val Tyr Pro Ala Asn Glu Val Thr
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Leu Leu Asp Ser Arg Ser Val Gln Gly Glu Leu Gly Trp Ile Ala Ser
35 40 45

Pro Leu Glu Gly Gly Trp Glu Glu Val Ser Ile Met Asp Glu Lys Asn
50 55 60

Thr Pro Ile Arg Thr Tyr Gln Val Cys Asn Val Met Glu Pro Ser Gln
65 70 75 80

Asn Asn Trp Leu Arg Thr Asp Trp Ile Thr Arg Glu Gly Ala Gln Arg
85 90 95

Val Tyr Ile Glu Ile Lys Phe Thr Leu Arg Asp Cys Asn Ser Leu Pro
100 105 110

Gly Val Met Gly Thr Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Tyr Glu
115 120 125

Ser Asp Asn Asp Lys Glu Arg Phe Ile Arg Glu Asn Gln Phe Val Lys
130 135 140

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Ile Asp Thr Ile Ala Ala Asp Glu Ser Phe Thr Gln Val Asp Ile Gly
145                 150                 155                 160

Asp Arg Ile Met Lys Leu Asn Thr Glu Ile Arg Asp Val Gly Pro Leu
                165                 170                 175

Ser Lys Lys Gly Phe Tyr Leu Ala Phe Gln Asp Val Gly Ala Cys Ile
            180                 185                 190

Ala Leu Val Ser Val Arg Val Phe Tyr Lys Lys Cys Pro Leu Thr Val
        195                 200                 205

Arg Asn Leu Ala Gln Phe Pro Asp Thr Ile Thr Gly Ala Asp Thr Ser
    210                 215                 220

Ser Leu Val Glu Val Arg Gly Ser Cys Val Asn Asn Ser Glu Glu Lys
225                 230                 235                 240

Asp Val Pro Lys Met Tyr Cys Gly Ala Asp Gly Glu Trp Leu Val Pro
                245                 250                 255

Ile Gly Asn Cys Leu Cys Asn Ala Gly His Glu Glu Arg Ser Gly Glu
            260                 265                 270

Cys Gln Ala Cys Lys Ile Gly Tyr Tyr Lys Ala Leu Ser Thr Asp Ala
        275                 280                 285

Thr Cys Ala Lys Cys Pro Pro His Ser Tyr Ser Val Trp Glu Gly Ala
    290                 295                 300

Thr Ser Cys Thr Cys Asp Arg Gly Phe Phe Arg Ala Asp Asn Asp Ala
305                 310                 315                 320

Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro Leu Asn Leu Ile
                325                 330                 335
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Ser Asn Val Asn Glu Thr Ser Val Asn Leu Glu Trp Ser Ser Pro Gln
            340                 345                 350

Asn Thr Gly Gly Arg Gln Asp Ile Ser Tyr Asn Val Val Cys Lys Lys
        355                 360                 365

Cys Gly Ala Gly Asp Pro Ser Lys Cys Arg Pro Cys Gly Ser Gly Val
    370                 375                 380

His Tyr Thr Pro Gln Gln Asn Gly Leu Lys Thr Thr Lys Val Ser Ile
385                 390                 395                 400

Thr Asp Leu Leu Ala His Thr Asn Tyr Thr Phe Glu Ile Trp Ala Val
                405                 410                 415

Asn Gly Val Ser Lys Tyr Asn Pro Asn Pro Asp Gln Ser Val Ser Val
            420                 425                 430

Thr Val Thr Thr Asn Gln Ala Ala Pro Ser Ser Ile Ala Leu Val Gln
        435                 440                 445

Ala Lys Glu Val Thr Arg Tyr Ser Val Ala Leu Ala Trp Leu Glu Pro
    450                 455                 460

Asp Arg Pro Asn Gly Val Ile Leu Glu Tyr Glu Val Lys Tyr Tyr Glu
465                 470                 475                 480

Lys Asp Gln Asn Glu Arg Ser Tyr Arg Ile Val Arg Thr Ala Ala Arg
                485                 490                 495

Asn Thr Asp Ile Lys Gly Leu Asn Pro Leu Thr Ser Tyr Val Phe His
            500                 505                 510

Val Arg Ala Arg Thr Ala Ala Gly Tyr Gly Asp Phe Ser Glu Pro Leu
        515                 520                 525
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Glu Val Thr Thr Asn Thr Val Pro Ser Arg Ile Ile Gly Asp Gly Ala
    530                 535                 540

Asn Ser Thr Val Leu Leu Val Ser Val Ser Gly Ser Val Val Leu Val
545                 550                 555                 560

Val Ile Leu Ile Ala Ala Phe Val Ile Ser Arg Arg Arg Ser Lys Tyr
                565                 570                 575

Ser Lys Ala Lys Gln Glu Ala Asp Glu Glu Lys His Leu Asn Gln Gly
            580                 585                 590

Val Arg Thr Tyr Val Asp Pro Phe Thr Tyr Glu Asp Pro Asn Gln Ala
        595                 600                 605

Val Arg Glu Phe Ala Lys Glu Ile Asp Ala Ser Cys Ile Lys Ile Glu
    610                 615                 620

Lys Val Ile Gly Val Gly Glu Phe Gly Glu Val Cys Ser Gly Arg Leu
625                 630                 635                 640

Lys Val Pro Gly Lys Arg Glu Ile Cys Val Ala Ile Lys Thr Leu Lys
                645                 650                 655

Ala Gly Tyr Thr Asp Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser
            660                 665                 670

Ile Met Gly Gln Phe Asp His Pro Asn Ile Ile His Leu Glu Gly Val
        675                 680                 685

Val Thr Lys Cys Lys Pro Val Met Ile Ile Thr Glu Tyr Met Glu Asn
    690                 695                 700

Gly Ser Leu Asp Ala Phe Leu Arg Lys Asn Asp Gly Arg Phe Thr Val
705                 710                 715                 720
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Ile Gln Leu Val Gly Met Leu Arg Gly Ile Gly Ser Gly Met Lys Tyr
725 730 735

Leu Ser Asp Met Ser Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
740 745 750

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Met Ser
755 760 765

Arg Val Leu Glu Asp Asp Pro Glu Ala Ala Tyr Thr Thr Arg Gly Gly
770 775 780

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ala Tyr Arg Lys
785 790 795 800

Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val Met Trp Glu
805 810 815

Val Met Ser Tyr Gly Glu Arg Pro Tyr Trp Asp Met Ser Asn Gln Asp
820 825 830

Val Ile Lys Ala Ile Glu Glu Gly Tyr Arg Leu Pro Pro Pro Met Asp
835 840 845

Cys Pro Ile Ala Leu His Gln Leu Met Leu Asp Cys Trp Gln Lys Glu
850 855 860

Arg Ser Asp Arg Pro Lys Phe Gly Gln Ile Val Asn Met Leu Asp Lys
865 870 875 880

Leu Ile Arg Asn Pro Asn Ser Leu Lys Arg Thr Gly Thr Glu Ser Ser
885 890 895

Arg Pro Asn Thr Ala Leu Leu Asp Pro Ser Ser Pro Glu Phe Ser Ala
900 905 910

Val Val Ser Val Gly Asp Trp Leu Gln Ala Ile Lys Met Asp Arg Tyr
915 920 925

Lys Asp Asn Phe Thr Ala Ala Gly Tyr Thr Thr Leu Glu Ala Val Val
930 935 940

His Val Asn Gln Glu Asp Leu Ala Arg Ile Gly Ile Thr Ala Ile Thr
945 950 955 960

His Gln Asn Lys Ile Leu Ser Ser Val Gln Ala Met Arg Thr Gln Met
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Gln Gln Met His Gly Arg Met Val Pro Val
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